EFFECT OF LOW DOSE RATE RADIATION ON CELL GROWTH KINETICS

EARLE C. GREGG, TOM M. YAU, AND S. C. KIM, *Division of Radiation Biology, Department of Radiology, Case Western Reserve University, Cleveland, Ohio 44106 U.S.A.*

ABSTRACT Experimental determinations were made of cell number as a function of time for two strains of L5178Y mammalian cells maintained continuously in various environments of radiation. One strain possessed a shoulder in its dose response curve whereas the other did not. Neither strain showed any significant difference in growth rate for interdivision doses on the order of the median lethal dose or less delivered continuously at a low dose rate or pulsed every 4 h at a high instantaneous dose rate. It was also shown that large numbers of dead cells have little effect on growth rate and that these dead cells last as discrete entities for many days. A simple theory of growth rate in the presence of radiation is presented, and the agreement with the observations implies that there is no effect of any sublethal low dose rate radiation received in one generation on the growth rate or radiation sensitivity of the succeeding generation. Further analysis of the data also showed that for the no-shoulder cells at 37°C, tritiated water had a relative biological effect close to unity for cell sterilization.

INTRODUCTION

In determining the rate of production of radiation-resistant mutations of mammalian cells when they were growing in a variety of radiation environments, it became obvious that the kinetics of cellular growth for the various radiation conditions had to be understood and involved in any attempt to quantitate the absolute mutation rate. Besides the intensity of radiation and the method of delivery, the growth rates of any particular cell line are also dependent on the quality and quantity of nutrition available (Kiefer et al., 1977), the temperature, and the actual mechanical handling of the cells (Adams et al., 1972; Nias and Lajtha, 1964). As will be described later, proper control of these parameters can lead to reasonably stable growth and repeatability of data under various radiation environments.

CELL LINES AND EXPERIMENTAL CONDITIONS

For the cells used in these experiments, we selected two strains of L5178Y mouse lymphoblasts: one with and one without a shoulder response to acute radiation exposure as shown in Fig. 1. Their radiation response data are tabulated in Table I. All symbols have their usual meaning (Hall, 1978*a*).

The "S/F" strain was originally isolated by J. T. Lett et al. (1964) and further reported by U. Ehmann et al. (1974). The terminology was changed from "S/S" originally used by Lett to "S/F" to indicate growth in our particular medium. The R_1C cells were cloned from a more resistant strain obtained from C. S. Lange at the University of Rochester, New York. The shoulder response in R_1C may be interpreted as being due to time-dependent repair of sublethal radiation damage that is

Dr. Yau is a Harry H. Pinney Scholar for Cancer Research at Case Western Reserve University.

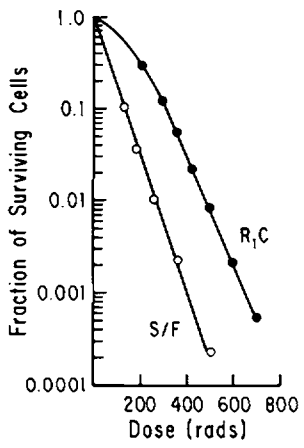

FIGURE 1

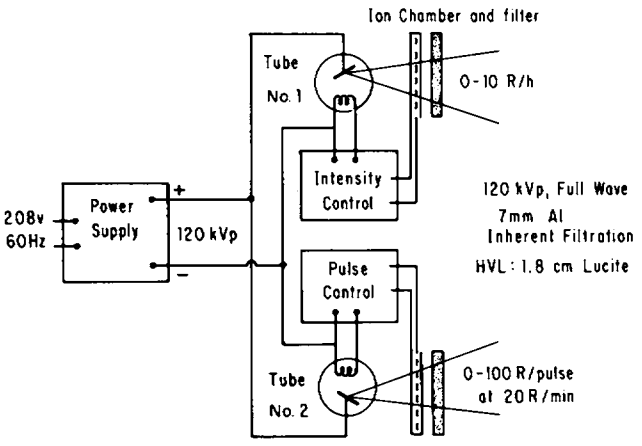

FIGURE 2

FIGURE 1 Response of two strains of L5178Y mouse lymphoblasts to single short-time (acute) exposures of x rays.

FIGURE 2 Schematic of the x-ray system that delivers both continuous and pulsed 120-kVp x rays. Ion chamber feedback is used to maintain a preset intensity in the continuous case and to deliver a constant dose per pulse in the second case.

apparently lacking in the more sensitive S/F strain.[1] All of these cells were maintained in static culture at 37°C in Fisher's medium containing 10% horse serum at densities ranging from 0.5 to 5.0 × 10^5 cells/ml. The serum content was increased to 15% for the chronically irradiated cells because they seemed to need a richer supply of nutrients for stability of growth rate. Plating experiments were done on Fisher's medium containing 20% horse serum plus 0.15% Difco Noble agar (Difco Laboratories, Detroit, Mich.) and maintained in a 3% CO_2 incubator for 10 d at 37°C. Plating efficiencies ranged from 60 to 80% for unirradiated cultures.

Cinephotomicrographic experiments to determine the interdivision times of surviving cells under continuous radiation conditions were performed with all the cells in a medium containing tritiated water and 0.075% Noble agar to restrict mobility. A Kodak Special II Camera (Eastman Kodak Co., Rochester, N.Y.) mounted on a Bausch & Lomb microsocpe (Bausch & Lomb Inc., Rochester, N.Y.) in a 37°C, walk-in incubator was used and the film advanced one frame every minute. The visual signal used for scoring was the appearance of a complete dark-line boundary between the two daughter cells as they were formed from the parent cell. This process took only 2–3 min to complete, which is a measure of the accuracy of the determination. Observations of such cell divisions were made over a ~48-h period. Control runs were made without tritiated water. Later replay and analysis of the films of those cells surviving the radiation led to a determination of the average interdivision time of cells in a particular bath of radiation. It must be emphasized that the doubling time of a culture is equal to the average interdivision time of the individual cells only when no sterile daughter cells are created. If incomplete divisions are produced by any agents or genetic defects, the doubling time is longer than the interdivision time.

Fig. 2 shows the experimental set-up used to produce both continuous and pulsed x-ray irradiations also located in a walk-in incubator. Up to 50 culture tubes can be irradiated simultaneously at various dose rates controlled by both filament temperature and distance from the target. Stability of the continuous irradiation intensity is maintained by feedback from a transmission ion chamber to the x-ray tube filament. In the pulsed source a similar ion chamber is used with an integrating circuit to deliver a

[1]Yau, T. M. Manuscript in preparation.

TABLE I

AVERAGE VALUES FOR THE RESPONSE OF TWO DIFFERENT L5178Y MUTANTS TO ACUTE DOSES OF X RADIATION

Strain	D_o	LD_{50}	n	D_q
	rads	*rads*		*rads*
S/F (without shoulder)	60	50	1.2	9.0
R_1C (with shoulder)	90	140	5.0	120

constant predetermined dose in each pulse. The culture tubes are moved continuously and circularly in the field to assure uniform radiation conditions and the intensity determined by both TLD and calibrated ionization chambers. Cultures have been maintained under these conditions for up to 6 mo. Continuous exposure to radiation seems to make the cells quite fragile to mechanical shock and sensitive to medium depletion. The condition of almost "zero" growth at ~5 rads/h for the S/F strain was particularly difficult to achieve. To maintain stability in the growth, these cultures had to be resuspended with great care in fresh medium every 2–3 d. If not, they would eventually cease to divide. Fig. 3 shows some typical growth curves of the S/F line under continuous x irradiation. Similar curves under similar conditions have been obtained by others (Nias and Lajtha, 1964; Okumura and Uchiyama, 1974; Szechter et al., 1978).

INITIAL OBSERVATIONS

Regarding mathematical analysis of the dynamics of growth of the above cells in culture while under continuous radiation, two experimental observations are worthy of note: (*a*) cells sterilized (or "killed") by radiation do not disappear for several days after exposure and (*b*) time lapse photography of the radiation sensitive strain (S/F) under continuous radiation levels ranging from 4 to 5 rads/h showed at most a 17–20% lengthening of the average interdivision time. Cell number as detected by a Coulter counter is plotted as a function of time in Fig. 4 for the two different strains following the listed single pulses of radiation. There is obviously some increase in number (~50%) due to mitoses immediately after irradiation

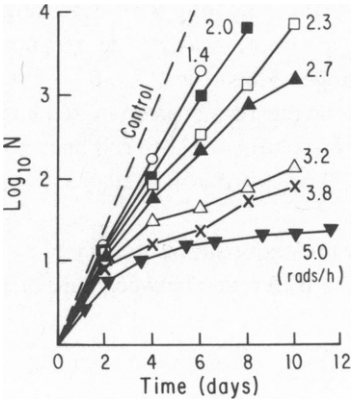

FIGURE 3

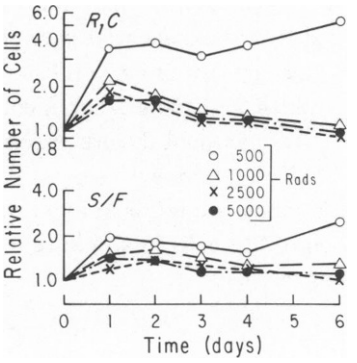

FIGURE 4

FIGURE 3 Typical growth curves for the S/F strain exposed to various dose rates of x irradiation.
FIGURE 4 Cell number determined by Coulter counting as a function of time after the listed single exposure doses of radiation.

which is then followed by a reasonably constant but small decline. Cell debris becomes noticeable after ~3 d as the number of dead cells diminishes, but this does not appreciably affect the analysis made later because of the exponential growth of the remaining live cells. The heavily irradiated sterilized cells appear slightly smaller and with darker edges than the original cells under phase microscopy. The few mitoses and the lingering dead cells correspond to the B and C cells assumed by Fox and Gilbert (1966) in their analysis of cell kinetics.

Analysis of the cinephotomicrographic films showed an increase in interdivision time from 10.5 ± 1.5 to 12.5 ± 2.3 h for a dose rate of 5 rads/h delivered continuously over 4 generations. This is an increase of 2 ± 0.4 h which is significant at a ~10% confidence level and indicates that radiation did have a small effect on the interdivision time. This delay disappears when the radiation is removed. Previous observations with acute doses of radiation (Rosenberg et al., 1976) established that $\Delta T = STD$, where ΔT is the mitotic delay induced by acute dose, D, when the culture has a doubling time, T, without radiation. S is $\simeq 5.9 \times 10^{-3}$ for S/F and $\simeq 0.9 \times 10^{-3}$ for R_1C. Using these data we find $\Delta T \simeq 2.6$ h for an acute median lethal dose (LD_{50}) of 50 rads for S/F and 1.1 h for the R_1C strain exposed to a LD_{50} dose of 140 rads. Data by Caldwell et al. (1965) support the calculation for the S/F strain. These calculations of mitotic delays with their attendant (but unknown) errors imply that there is little difference between division delay in S/F due to continuous radiation integrated over one interdivision time and that due to an acute dose of the same magnitude. Further, it would appear that division delay is almost negligibly small in R_1C for doses on the order of LD_{50}.

GROWTH KINETICS

We shall now assume that growth and response to radiation are exponentially related to time. Namely, for growth $dN/dt = \alpha N$ and, for lethality $dN/dt = -\beta RN$, where N is the number of live cells at any time t, α is the fractional rate of growth of cells surviving the radiation, R the dose rate and β the fractional number killed per unit dose of exposure. It is obvious that the second expression does not hold exactly for the R_1C strain because of the presence of a shoulder in its response curve. However, since we shall be dealing with doses slightly larger than the LD_{50}, we shall make the simplifying assumption that the response may be approximated by an exponential over this dose range. Thus, for R_1C, $\beta = \ell n(2)/LD_{50} = 0.005$/rad. This approximation will have little effect on our results as discussed later. For S/F we have $\beta = 0.014$/rad and measurement of the doubling time for both cell lines produced $\alpha = \ell n(2)/T \sim 0.07$/h without the presence of radiation. For 19% division delay as observed at ~5 rads/h for the S/F strain, we have $\alpha \sim 0.06$.

Under continuous radiation at any one time with the above simplifying conditions, the rate of production of live cells in a culture is equal to the difference between rate of growth and rate of killing, namely:

$$dN_L/dt = \alpha N_L - \beta R N_L. \tag{1}$$

The solution of this equation is, of course,

$$N_L = N_0 \exp(\alpha - \beta R)t, \tag{2}$$

where N_0 is the number of cells at $t = 0$.

Now the rate of only killing cells at any time t is:

$$\frac{dN_D}{dt} = \beta R N_L = \beta R N_0 \exp(\alpha - \beta R)t, \tag{3}$$

from which the number of dead cells at any time t becomes

$$N_D = \frac{1.5 \beta R N_0}{\alpha - \beta R}(e^{[\alpha - \beta R]t} - 1), \tag{4}$$

where the factor 1.5 is due to the divisions still occurring after a lethal dose of radiation as shown previously.

Because a Coulter counter will count both live and dead cells, the Coulter count of a culture irradiated at a rate R rads/h becomes

$$N = N_L + N_D = N_0 e^{(\alpha - \beta R)t} + \frac{1.5 \beta R N_0}{\alpha - \beta R}(e^{[\alpha - \beta R]t} - 1), \tag{5}$$

or

$$N/N_0 = e^{(\alpha - \beta R)t}\left(1 + \frac{1.5 \beta R}{\alpha - \beta R}\right) - \left(\frac{1.5 \beta R}{\alpha - \beta R}\right). \tag{6}$$

It is to be noted that one assumption in the above solution is that α remains constant over the observation period. That is, neither the presence of dead cells nor variations in mitotic delay will appreciably affect the growth rate. One other assumption is that β is constant and independent of past exposure history for each cell and that each newborn cell has no memory of any sublethal dose received by its parent cells. This assumption is partially supported by the opinion expressed by Lamerton and Courtenay (1969) that if significant radiation effects at low dose rates are single lethal events, then the total radiation dose delivered during the cell cycle is a more important factor than dose rate in explaining growth kinetics under continuous irradiation conditions.

It is important to note that the chance of sterilization for the S/F strain from an acute dose of radiation is $\sim 10^{-2}$/rad, whereas the chance of producing a mutant (or variant) is $\sim 2 \times 10^{-8}$/rad so that the chance of a special mutant appearing and perturbing experimental observations is relatively remote.

For times such that $e^{(\alpha - \beta R)t} > 1$, Eqs. 5 and 6 show a slope of $(\alpha - \beta R)$ when log N is plotted against time. This, of course, is a new growth rate $\alpha' = \alpha - \beta R$. This expression also predicts a condition of zero growth or a stationary growth as $\beta R \rightarrow \alpha$, which condition was first observed by Courtenay (1965, 1969) for $R = 4.8$ rads/h with her "normal" L5178Y cells, which had a common origin with our S/F strain. Although for $\alpha = \beta R$ the growth rate equals the rate of sterilization, which implies zero growth, there is a steady increase in counted cells for a time after the start of the experiment due to the accumulation of dead cells. However, an apparent zero growth condition will still be achieved when the total number of cells disappearing per unit time by lysing at some later time equals the number sterilized at that time. This can take up to a week after the start of the observations and the live:dead cell ratio can be small depending on the rate of disappearance of the sterilized cells. Obviously, shorter times are required for stable growth rates with lower dose rates. These delays in achieving equilibrium

have not yet been measured accurately. It is rather important to point out that the overall growth rate ($\alpha-\beta R$) is very sensitive to small changes in α, β, and R, particularly near zero growth conditions.

Converting the parameters α and β to doubling times and acute doses, one obtains:

$$\frac{1}{T_{dc}} = \frac{1}{T} - \frac{R}{D_{50}}, \tag{7}$$

where T_{dc} is the doubling time of the continuously irradiated culture, T is the interdivision time of the original cells including mitotic delay if necessary, D_{50} is the acute dose in rads required to produce 50% sterility, and R is the dose rate. A plot of R vs. $1/T_{dc}$ should then produce a straight line with a negative slope.

Numerous growth curves similar to those in Fig. 3 were determined for the R_1C strain exposed to various intensities of continuous 120-kV x irradiation and for the S/F strain exposed to continuous and pulsed 120 kV radiation and tritiated water in the culture medium. From the slopes of the straight line portions of the growth curves, one then obtains T_{dc}. Fig. 5 is a plot of $1/T_{dc}$ vs. R for the two strains of mammalian cells for a variety of exposures. The straight lines shown connect a predetermined point on each axis. The one shown on the abscissa is obviously related to α, which is very closely the same for both strains of cells. The other points on the ordinate for each mutant are those dose rates calculated from the acute exposure data ($R = \alpha/\beta$), which will theoretically produce equilibrium growth. The value of $R = 5.0$ rads/h for the S/F strain is calculated from the acute data which rate should drop to 4.1 rads/hour if all the expected mitotic delay were introduced. The value of $R = 14.0$ rads/h

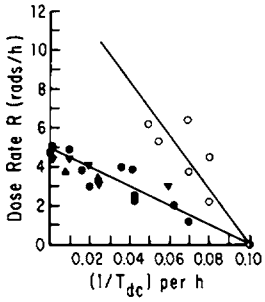

FIGURE 5

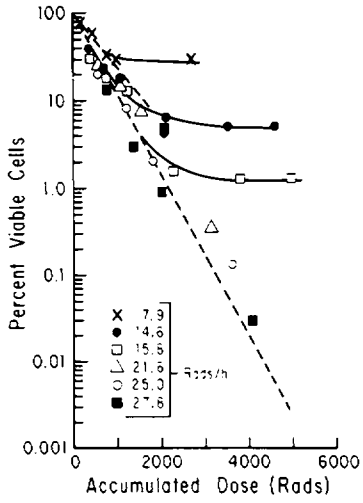

FIGURE 6

FIGURE 5 Plot of dose rate vs. the reciprocal of culture doubling time under protracted irradiation. O, subline R_1 continuously irradiated with 120-kVp x rays; ●, subline S/F continuously irradiated with 120-kVp x rays; ▼, subline S/F irradiated with 120-kVp x rays once every 4 h; ▲, subline S/F in tritium; ■, subline *N* in tritium (from Courtenay).

FIGURE 6 Number of viable R_1C cells as a function of accumulated exposure dose ($D = Rt$) for various dose rates (R).

was calculated for the R_1C strain for which mitotic delay is negligible. The experimental points shown on the ordinate axis are the averages of many observations on the S/F strain for exposures to tritiated water (our own data as well as Courtenay's), continuous 120 kV x-rays and continuously pulsed, 120-kV x rays. The very satisfactory agreement of these points on the ordinate axis indicate that all three radiations have the same lethal effect to within 10%. Since the RBE of 120-kV x-rays is very near unity, this is tantamount to concluding that the RBE of tritiated water for inducing sterility is also unity. For the intermediate experimental points, the errors in determining the growth rate from the slopes of the growth curves increased relative to the data on the ordinate axis because of the difficulty in determining the time at which to measure the slope. This difficulty was also compounded by the outgrowth of radiation-resistant mutations. Nevertheless, the clustering of the data points about the lines indicates reasonable agreement with theory, which in turn neglects any division delay due to the radiation and possibly different growth rates due to the presence of dead cells. The dosimetry for tritiated water was based on the relation: dose (rads per hour) = (0.0096)(microcuries per milliliter). Higher values of RBE for the lethality of the exposure of V79 and L5178Y cells to tritiated water have been reported by Bedford et al. (1975), but the experimental circumstances were different from those reported here in that the cells were exposed in the frozen state at 5°C.

It is to be noted from the previous expressions that the dead cell:live cell ratio may be written as:

$$\frac{N_D}{N_L} = \frac{\dfrac{1.5\,\beta R}{\alpha - \beta R}\left(e^{[\alpha-\beta R]t} - 1\right)}{e^{(\alpha-\beta R)t}} \approx \frac{1.5\,\beta R}{\alpha - \beta R} \tag{8}$$

for $\alpha > \beta R$ and t large enough that $e^{(\alpha-\beta R)t} \gg 1$. For $\alpha \approx \beta R$, we find that $N_D/N_L \rightarrow 1.5\,\beta Rt$, which means that the dead cells will overwhelm the live cells which will seemingly disappear when trying to maintain nearly zero growth conditions. For dose rates slightly lower than $R = \alpha/\beta$, dead:live cell ratios of 20–30 are quite achievable and allow seemingly normal growth.

If one now counts only the live cells by plating but normalizes to the Coulter count, which measures both live and dead cells at the time of sampling, we obtain for the fraction of viable cells:

$$\frac{N_L}{N_L + N_D} = \rho = \frac{1}{1 + \dfrac{1.5\,\beta R}{\alpha - \beta R}\left(1 - e^{-[\alpha-\beta R]t}\right)}, \tag{9}$$

which for $\alpha > \beta R$ and large t becomes a constant:

$$\rho \simeq \frac{\alpha - \beta R}{\alpha + 0.5\,\beta R}. \tag{10}$$

This appears as a "plateau" when log ρ is plotted against time or accumulated dose. For small $(\alpha - \beta R)t$, we find

$$\rho \simeq e^{-1.5\,\beta Rt} = e^{-1.5\,\beta D}, \tag{11}$$

where D is the accumulated dose Rt. This obviously produces a straight line when log ρ is plotted against D or t.

For $\beta R > \alpha$, where the dose rate is greater than that required for zero growth and which produces a positive exponential in Eq. 9, we see

$$\rho = \frac{1}{1 + \dfrac{1.5\,\beta R}{\beta R - \alpha}\,(e^{[\beta R - \alpha]t} - 1)}. \tag{12}$$

For very small $(\beta R - \alpha)t$, this becomes again

$$\rho \simeq e^{-1.5\beta Rt} = e^{-1.5\beta D}, \tag{13}$$

whereas, for large $(\beta R - \alpha)t$,

$$\rho \approx \frac{\beta R - \alpha}{1.5\,\beta R}\,e^{-(\beta R - \alpha)t}. \tag{14}$$

Fig. 6 shows the experimentally determined ratio ρ for the strain R_1C as a function of accumulated dose Rt for various values of R. From Eq. 10 we find for the plateau values 38, 7, and 2%, which are in reasonable absolute agreement with the data shown in Fig. 6. From the slope of the data at small t for R = 7.9 rads/h and Eq. 11, we calculate β = 0.004, which is again in agreement with data from the acute dose response curve. From the data at large doses

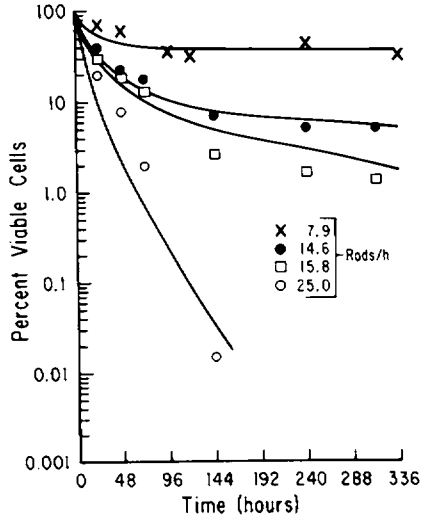

FIGURE 7

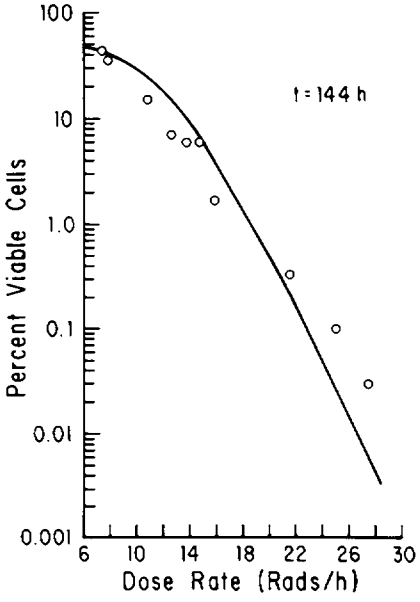

FIGURE 8

FIGURE 7 Number of viable R_1C cells as a function of time (t) for various dose rates (R). The solid curves are calculated from Eqs. 9 and 12, whereas the points are experimental determinations.

FIGURE 8 Number of viable R_1C cells 144 h after the start of the exposure as a function of the dose rate R. Solid curve is calculated from Eq. 9, and the points are experimental determinations.

for R = 27.8 rads/h and Eq. 14, we find β = 0.005. Fig. 7 is a replot of most of the data in Fig. 6 but with time as one axis instead of accumulated dose D. The solid curves are calculated from Eqs. 9 and 12 and the constants given in Table I. The solid lines in Figs. 8 and 9 show ρ vs. R for given values of t in Eq. 9, whereas the points are experimental data.

Fig. 10 presents experimentally obtained data for the S/F strain, which shows results for both continuous and pulsed radiation at ~5 rads/h. Independence of mode of delivery might be expected due to lack of repair of sublethal damage in this particular strain. Regardless, the solid line is the best fit of Eq. 9 with β = 0.028 and $\alpha - \beta R \sim 0.001$. This apparently doubled radiosensitivity as seen in the value for β may be explained by the effect of a possible pile-up of cells in G_2/M due to the larger mitotic delay for S/F than occurs in R_1C. This would be noticeable at the short times involved (<180 h) with a decreasing β occurring at the longer times as implied by the last points shown. This effect of pile-up in G_2/M on radiosensitivity has recently been emphasized by Hall (1978*b*). For lower dose rates, plateau values of ρ for the S/F strain as shown in Table II agree reasonably well with values calculated from Eq. 10 with β = 0.014.

Ignoring the effect of pile-up in the S/F strain at short times, the agreement between theory and experiment for both mutant lines shows that both α and β are reasonably constant for at least 18 d (40 generations). This indicates that the presence of dead cells has little effect on α and that sublethal doses of radiation received in one generation of cells has little or no effect on the succeeding generations.

Fig. 11 shows the response of R_1C cells as a function of time to two intensities of continuous and continuously pulsed (six times daily) radiations. Although the data indicates that for the same daily dose the continuous radiation is more effective than the pulsed in killing cells, not so obvious is the fact that the difference is actually small. β as evaluated from both Eq. 10 for R = 9 rads/h and Eq. 14 for R = 20 rads/h is 0.008 for the pulsed radiation and 0.009 for continuous radiation—a difference of only 12%. Considering the possible changes in α due to

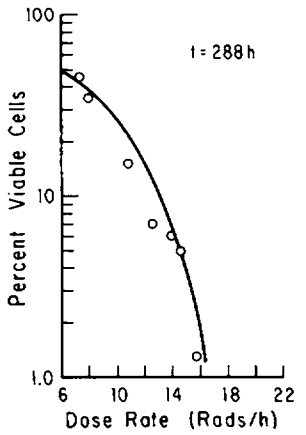

FIGURE 9

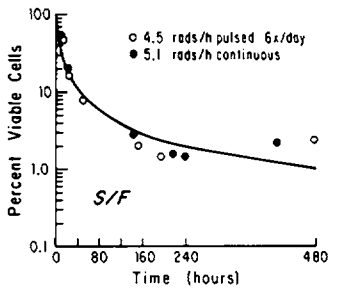

FIGURE 10

FIGURE 9 Number of viable R_1C cells 288 h after the start of the exposure as a function of dose rate R. Solid curve is calculated from Eq. 9, and the points are experimental determinations.
FIGURE 10 Viable S/F cells as a function of time for the dose rates shown.

TABLE II

VALUES OF ρ FOR THE S/F STRAIN UNDER PROTRACTED RADIATION

Dose rate	Plateau ρ	
	Calculated	Measured
rads/h		
1.4	0.58	0.52
2.0	0.45	0.40
2.3	0.38	0.27
2.7	0.30	0.19
3.2	0.20	0.12
3.3 (Pulsed)	0.19	0.06
3.6 (Pulsed)	0.14	0.06
3.9	0.08	0.09
4.5 (Pulsed)	0	0.02

the different modes of delivery of radiations, the changes in β due to cell cycle pile-up, and the possibility of errors in absolute dose measurement of the radiations, it is our opinion that this difference is not significant. If such is true, the implication is that appreciable time-dependent repair does not occur for doses below the LD_{50} for R_1C and that the overall response is primarily dependent on the initial slope of the dose response curve.

SUMMARY

Measurements of changes in density of cultured L5178Y cells under various conditions of environmental radiation show that simple exponentials for cell growth and sterilization are adequate to explain most of the kinetics involved. These data also indicate the following. (*a*) Dead:live cell ratios ranging up to 20 or 30 have little effect on the growth rates of the live cells. (*b*) Sterilized, or dead cells, remain in suspension as well-defined entities for several days. (*c*) L5178Y cells with and without a shoulder in their dose response curves demonstrate little or no difference between the effects of radiation delivered continuously or pulsed every 4

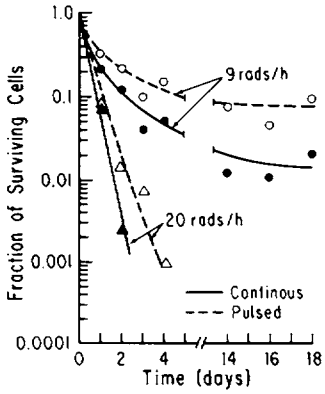

FIGURE 11 Viable R_1C cells as a function of time for two different dose rates and both continuous and pulsed sources of radiation. The pulses were delivered every 4 h at the daily average rate shown.

h with the same average dose rate provided the total exposure dose over one interdivision time interval does not appreciably exceed the LD_{50}. (*d*) Tritiated water has a RBE close to unity for sterilization of L5178Y cells in suspension at 37°C. (*e*) Division delay due to interdivision exposure doses on the order of the LD_{50} or less from continuous or continuously pulsed radiation apparently remains constant for successive generations of cells exposed to the same radiation environment. This delay returns to zero when the radiation is removed. (*f*) For over at least 40 generations there was no indication in terms of lethality that any sublethal dose of radiation received in one generation of cells had an effect on the succeeding generation.

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